Remarks

I. Status and Nature of the Invention

Claims 1-95 were originally presented. Claims 1-4 and 56-95 have been withdrawn as directed to non-elected inventions. Claims 15, 21 and 22 have been cancelled. Accordingly claims 5-14, 16-20, and 23-55 are presently pending. In light of the Examiner's request for an election of species, Applicants have elected as an enzyme, the enzyme: **caspase**, and as an uptake-enhancing agent, the uptake-enhancing agent: **glycerol**, and as an indicator moiety, the indicator moiety: **rhodamine 110**. Applicants appreciate the decision of the Examiner to examine simultaneously the species of glycerol (reading on claims 5-19 and 38-55) and DMSO (reading on claims 20-22).

Applicants have amended claim 5 in order to more clearly recite that the recited assay is to be conducted in living and metabolically active cells. Support for such an amendment can be found throughout the specification (see, for example, page 14, line 21 – page 15, line 18. Claim 5 has also been amended to recite the uptake-enhancing agents that had been previously recited in claim 15. Support for this amendment can be found, for example, in claims 15 and 22. Claim 13 has been amended to correct an obvious typographical error. No new matter has been entered by any of the requested amendments.

The present invention relates to an improved method for conducting enzyme assays in which the assay is conducted within living and metabolically active cells. In accordance with a preferred embodiment of the invention, cells are incubated with a substrate or analyte for an enzyme that is to be assayed in the presence of an agent that enhances the uptake of that substrate or analyte into the metabolically active cells. The enzyme assay is then conducted while such cells are alive and metabolically active.

II. The Rejections Pursuant to 35 U.S.C. § 112, Second Paragraph

Claims 9, 10, 54, and 55 have been rejected pursuant to 35 U.S.C. §112, second paragraph as being indefinite out of a concern that the recitations of "mixed" and "unmixed" reagents would be considered confusing by those of ordinary skill in the art in light of the recitations of claim 5. Applicants respectfully traverse the rejection and request reconsideration.

Applicants respectfully submit that the present invention relates to assays that are conducted on metabolically active cells. Claim 5 recites that such assay is to be conducted "in the presence of an agent that enhances uptake of said substrate or analyte." As the Examiner will also note, claim 5 thus requires no more than the *co-presence* of the substrate or analyte compound and the uptake-enhancing agent. The invention, however, comprises two preferred sub-embodiments. In the first such sub-embodiment, the substrate or analyte compound and the uptake-enhancing agent are not merely "co-present," but are mixed together during the assay. In the second such sub-embodiment, the substrate or analyte compound and the uptake-enhancing agent are not mixed. The Examiner contends that the terms "mixed" and "unmixed" are unclear, and thus renders the Claims 10 and 55 confusing. Applicants respectfully submit that those of ordinary skill would fully understand the metes and bounds of the "mixed" and "unmixed" terms. In this regard, Applicants respectfully draw Examiner's attention to page 8, line 16 of the Application where such conditions are discussed. In addition, Applicants respectfully draw the Examiner's attention to page 47, lines 17-22 of the present application in which Applicants provide detailed explanations of these terms.

As disclosed in the specification, the difference in incubation conditions (mixed vs. unmixed) has been found to confer marked advantages on assay results, depending upon the substrate/analyte and uptake-enhancing agent employed. In this regard, the Examiner is invited to review Figures 1-3, and the legends of these Figures appearing at page 46 of the Specification.

Applicants therefore respectfully submit that the rejection of claims 9, 10, 54 and 55 pursuant to the second paragraph of 35 U.S.C. §112(b) as being indefinite may be properly withdrawn.

III. The Rejections Pursuant to 35 U.S.C.§ 102

A. The Rejections in View of Los *et al.*

Claims 5-9, 13 and 14 have been rejected pursuant to 35 U.S.C. §102(b) as anticipated by Los *et al.* (Nature 375:81-83(1995)). Applicants respectfully traverse the rejection and request reconsideration.

Applicants respectfully submit that the claimed invention is not anticipated by the disclosure of Los *et al.* As the Examiner will appreciate, the present invention employs an uptake-facilitating agent and relates to the conducting of an enzyme assay in metabolically active cells. The cited Los *et al.* reference discloses a method of permeabilizing a cell with 0.05% digitonin in order to enable the entry of a fluorogenic substrate (please see page 82, Figure 1 legend). Since digitonin is known to be toxic to cells (see, for example, Szydlowska, H. *et al.* "MEMBRANOLYTIC ACTIVITY OF DETERGENTS AS STUDIED WITH CELL VIABILITY TESTS," Folia Histochem Cytochem (Krakow). 1978;16(2):69-78, which discloses that digitonin is much more toxic than the detergents, sodium dodecylsufate or Triton X-100), Applicants respectfully submit that those of ordinary skill would conclude that the cells disclosed in the Los *et al.* publication were not metabolically active. Applicants have additionally amended claims 5-9, 13 and 14, and respectfully submit that the rejection of claims 5-9, 13 and 14 pursuant to 35 U.S.C. §102(b) as anticipated by Los *et al.* (Nature 375:81-83 (1995)) may be properly withdrawn.

B. The Rejections in View of Wansink *et al.*

Claims 5, 9, 15-19 have been rejected pursuant to 35 U.S.C. §102(b) as anticipated by Wansink *et al.* (J. Cell Biol. 122(2):283-293(1993)). Applicants respectfully traverse the rejection and request reconsideration.

Applicants respectfully submit that the claimed invention is not anticipated by the disclosure of Wansink *et al.* As the Examiner will appreciate, the claimed invention comprises treating cells with a substrate or analyte for an enzyme that is to be assayed in the presence of an agent that enhances the uptake of that substrate or analyte into the metabolically active cells, and then assaying the metabolically active cells for enzymic activity. In this regard, Applicants respectfully draw the Examiner's attention to step (b) in Claim 5, which states that Applicants' method assays metabolically active whole cells for changes in concentration of enzyme on substrate or analyte.

In contrast, the cited Wansink *et al.* reference discloses a method of permeabilizing a cell with 25% glycerol buffer in Triton X-100. The assay for enzymic activity is, however, not performed on metabolically active cells, but rather is performed on *extracellular precipitates* of cellular DNA obtained through the TCA-mediated lysis of the cells (please see page 284, left hand column, section entitled "BrUTP Incorporatation in Permeabilized Cells (Run-on Transcription)"). It is therefore submitted that the claimed method is as not practiced by Wansink *et al.*, and that Wansink *et al.* would thus not have anticipated the claims of the present invention.

Applicants, therefore, respectfully submit that the rejection of claims 5, 9, 15-19 pursuant to 35 U.S.C. §102(b) as anticipated by Wansink *et al.* (J. Cell Biol. 122(2):283-293(1993)) may be properly withdrawn.

C. The Rejections in View of Lucas *et al.*

Claims 5, 9, 13-15, 20, 21, 46 and 54 have been rejected pursuant to 35 U.S.C. §102(b) as anticipated by Lucas *et al.* (U.S. Patent 5,698,411). Applicants respectfully traverse the rejection and request reconsideration.

Applicants have amended the present claims to more clearly distinguish their invention from that of Lucas *et al.* Applicants note that Lucas *et al.* discloses solubilizing components, including DMSO at 5%, along with several clearly toxic detergents. It is

respectfully submitted that the Lucas *et al.* patent does not teach the use of concentrations of DMSO that exceed 5%. Applicants further draw the Examiner's attention to column 30, lines 40-50 of the Lucas *et al.* patent, wherein Lucas *et al.* teach that difficulties are encountered when using a solubilizing component, particularly in facilitating the expulsion of the substrate from the metabolically active cell. The patent teaches that such expulsion negatively affects assay sensitivity. It is therefore respectfully submitted that the amended claims are not anticipated by the Lucas *et al.* patent. Accordingly, Applicants respectfully submit that the rejection of claims 5, 9, 13-15, 20, 21, 46 and 54 pursuant to 35 U.S.C. §102(b) as anticipated by Lucas *et al.* (U.S. Patent 5,698,411) may now be properly withdrawn.

D. The Rejections in View of Zhang *et al.*

Claims 5, 9, 13-15, 38-40, 46, 48-51 and 54 have been rejected pursuant to 35 U.S.C. §§102(a) and/or (e) as anticipated by Zhang *et al.* (U.S. Patent 6,248,904). Applicants respectfully traverse the rejection and request reconsideration.

Applicants respectfully submit that Zhang *et al.* teaches the use of lipophilic solvents or vehicles for the purpose of enhancing the aqueous solubility of fluorogenic or fluorescent compounds. The patent identifies the following suitable solubilizers: water-soluble salts and alkaline solutions of the fluorogenic or fluorescent compounds, fatty oils, ("for example, sesame oil"), synthetic fatty acid esters ("for example, ethyl oleate, triglycerides"), polyethylene glycol-400 or dimethylsulfoxide (DMSO)). Applicants respectfully draw the Examiner's attention to the fact that beyond the mere listing of reagents, no guidance is provided as to the concentration or manner in which such reagents are used, and whether they are to be retained throughout the assay. Applicants respectfully submit that, particularly in light of the disclosure by Lucas *et al.* of the difficulties attendant in the use of solubilizing components (please see above), the mere mentioning of DMSO by Zhang *et al.* would not have placed the public in possession of the claimed invention, and that as such, the Zhang *et al.* Patent fails to anticipate the claimed invention.

Applicants have additionally amended the claims of the present invention, and respectfully submit that the amended claims are not anticipated by the Zhang *et al.* patent. Accordingly, Applicants respectfully submit that the rejection of claims 5, 9, 13-15, 38-40, 46, 48-51 and 54 pursuant to 35 U.S.C. §102 as anticipated by Zhang *et al.* (U.S. Patent 6,248,904) may now be properly withdrawn.

III. The Rejections Pursuant to 35 U.S.C.§ 103(a)

A. The Rejections in View of Lucas *et al.*

Claims 5, 11, 12 and 22 have been rejected pursuant to 35 U.S.C. §103(a) as obvious in view of Lucas *et al.* (U.S. Patent 5,698,411). The rejection is predicated on the concern that although Lucas *et al.* fails to disclose an assay in which multiple enzyme assays are conducted, or in which DMSO concentrations of between 20% and 60% are employed, such modifications would have been obvious to those of ordinary skill. Applicants respectfully traverse the rejection and request reconsideration.

Applicants respectfully submit that for Applicants' claims to multiple enzyme assays to be obvious, more is required than the mere motivation of the art to possess superior technology and the recognition that the blending of technologies, if feasible, would be nice. It is submitted that the law requires the specific elements absent from a reference be found in the prior art, and there be a specific teaching that such elements be combined with the approach taught by that reference.

In this regard, Applicants respectfully draw the Examiner's attention to column 30, lines 40-50 of the Lucas *et al.* patent, wherein Lucas *et al.* teach that difficulties are encountered when using a solubilizing component, particularly in facilitating the expulsion of the substrate from the metabolically active cell. The patent teaches that such expulsion negatively affects assay sensitivity. It is therefore respectfully submitted that the conducting of multiple enzyme assays in metabolically active cells as presently claimed is too complex an undertaking for those of ordinary skill to achieve based merely on their

motivation to succeed and the teachings of Lucas *et al.* It is respectfully submitted, therefore that the rejection of claims 5, 11, 12 and 22 on this basis may be properly withdrawn.

Applicants have amended the present claims to recite that if the uptake-enhancing agent employed is DMSO, then it be present at a concentration of between 20% and 60%. In this regard, Applicants respectfully submit that the use of higher concentrations of DMSO than those taught by Lucas *et al.* is accompanied by unexpected results. The Examiner's attention is therefore respectfully directed to **Figure 6B**, which shows a dramatic and unexpected increase in assay sensitivity with higher DMSO concentration. Applicants respectfully submit that such evidence rebuts any prima facie conclusion of the obviousness of employing DMSO at high concentrations as the uptake-enhancing agent, and establishes the non-obviousness of the invention, especially those claims directed to the use of DMSO for this purpose.

Accordingly, Applicants respectfully submit that the rejection of claims 5, 11, 12 and 22 pursuant to 35 U.S.C. §103(a) as anticipated by Lucas *et al.* (U.S. Patent 5,698,411) may now be properly withdrawn.

B. The Rejections in View of Landrum *et al.* in combination with Lucas *et al.*

Claims 5, 13 and 38-54 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over Landrum *et al.* (U.S. Patent 5,976,822) in view of Lucas *et al.* (U.S. Patent 5,698,411). The rejection is predicated on the concern that although Landrum *et al.* fails to disclose the use of uptake-enhancing agents, the inclusion of such additional ingredients would have been obvious to those of ordinary skill in light of the teaching of Lucas *et al.* that certain solubilizing agents can be employed in a cell-based enzyme assay. Applicants respectfully traverse the rejection and request reconsideration.

As discussed above, Applicants submit that Lucas *et al.* teach seven fluorescent dye solubilizing components, of which several are toxic and, thus incompatible with the objectives of the present invention – assaying enzyme(s) in a metabolically active cell. Applicants respectfully submit that the Landrum et al patent, in combination with the disclosure of Lucas *et al.*, fails to provide the requisite guidance needed to achieve the present invention. There is no motivation to avoid the toxic compounds taught by Lucas *et al.*, nor is there any motivation to employ higher concentrations of DMSO.

Additionally, it is submitted that Applicants' invention is attendant with indicia of unexpected results that rebut any conclusion of obviousness. The superior results obtained by the inventors with high concentrations of DMSO is, it is submitted, not obvious in light of the Lucas *et al.* patent.

Accordingly, Applicants respectfully submit that the rejection of claims 5, 13 and 38-54 pursuant to 35 U.S.C. §103(a) as obvious over Landrum *et al.* (U.S. Patent 5,976,822) in view of Lucas *et al.* (U.S. Patent 5,698,411) may now be properly withdrawn.

C. The Rejections in View of Zhang *et al.*

Claims 5-15, 20-22 and 38-54 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over Zhang *et al.* (U.S. Patent 6,248,904). The rejection is predicated on the concern that although Zhang *et al.* fails to disclose an assay in which multiple enzyme assays are conducted, or in which DMSO concentrations of between 20% and 60% are employed, such modifications would have been obvious to those of ordinary skill. Applicants respectfully traverse the rejection and request reconsideration.

As previously noted, the art recognized difficulties that arise from the use of solubilizers in enzyme assays are believed to preclude any inference that the claimed invention could have achieved without guidance as to the reagents, concentration and manner of provision for achieving enhanced uptake of compounds into metabolically

active cells. Thus, as one example, nothing in the cited art suggests that glycerol could be employed to increase assay sensitivity.

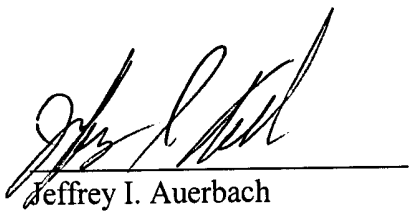
Applicants again respectfully submit that the use of high concentrations of DMSO is associated with unexpected improvements in assay sensitivity. Particularly in light of the complete silence of Zhang as to concentration or manner in which the Zhang *et al.* "solubilizers" are to be employed, it is submitted that the reference does not render the present invention obvious.

Accordingly, Applicants respectfully submit that the rejection of claims 5-15, 20-22 and 38-54 pursuant to 35 U.S.C. §103(a) as obvious over Zhang *et al.* (U.S. Patent 6,248,904) may be properly withdrawn.

III. Concluding Remarks

Having now fully responded to all outstanding rejections, Applicants respectfully submit that the present application is in condition for Allowance, and earnestly solicit early notice of such favorable action. The Examiner is respectfully invited to contact the undersigned with respect to any issues regarding this application.

Respectfully Submitted,



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